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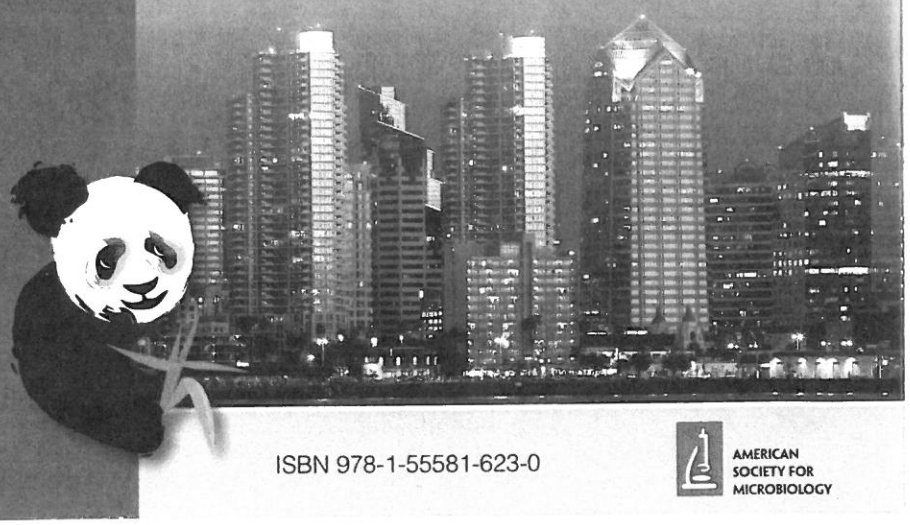

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DEPARTMENT OF VETERINARY DISEASE BIOLOGY
FACULTY OF LIFE SCIENCES
UNIVERSITY OF COPENHAGEN

Role of HtrA protease and chaperone activity in stress tolerance and virulence of *Campylobacter jejuni*

Kristoffer Torbjørn Bæk[1], Christina Skovgaard Vegge[1], Joanna Skórko-Glonek[2] and Lone Brøndsted[1*]

[1]Department of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen, Stigbøjlen 4, DK-1870 Frederiksberg C, Denmark
[2]Department of Biochemistry, University of Gdańsk, Kladki 24, 80-822 Gdańsk, Poland

Contact information*
E-mail: [redacted]
Phone: +45 35 33 27 64

Introduction

The food-borne human pathogen *Campylobacter jejuni* is the most common cause of bacterial food-borne infections in developed countries. *C.jejuni* is a microaerophilic bacterium with at narrow temperature interval for growth and an optimum temperature of 42°C.

Envelope stress tolerance in Gram negative bacteria relies on proteases as well as conserved chaperones such as HtrA, SurA, Skp, and FkpA to degrade or refold damaged periplasmic proteins. However, *C. jejuni* lacks homologs of the Skp and FkpA chaperones and HtrA may therefore play a more dominant role in this bacterium.

HtrA encodes both chaperone and protease activity, but little is known about how each of these activities contributes to stress tolerance and virulence in pathogenic bacteria.

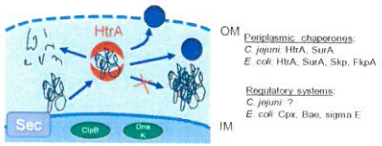
Objective

To determine the contribution of protease and chaperone activities of HtrA to heat and oxidative stress tolerance as well as virulence of *C. jejuni*.

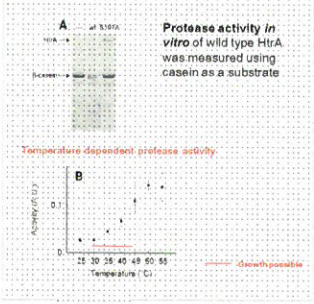
Conclusions

- HtrA of *C. jejuni* is able to degrade misfolded proteins and prevent formation of protein aggregates
- HtrA chaperone activity is sufficient to support growth during most conditions
- To tolerate higher degrees of stress the protease activity is needed for degradation of misfolded proteins or to empty the cavity of HtrA allowing proteins to be folded or transferred to the outer membrane
- The requirement of HtrA for growth under heat stress depends entirely on the level of oxidative stress
- The oxidative stress sensitivity of *htrA* mutants have no effect on survival in macrophages
- The chaperone activity may be involved in folding of virulence factors important for adherence and invasion. Currently it is unknown if HtrA degrades FlaA *in vivo* and how this may have an influence on virulence of *C. jejuni*

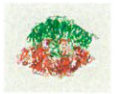
HtrA in *C. jejuni*



HtrA is a protease...

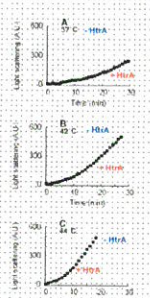


Protease activity *in vitro* of wild type HtrA was measured using casein as a substrate



HtrA and the proteolytically inactive variant, **$HtrA_{S197A}$**, was purified by nickel affinity chromatography.

...and a chaperone



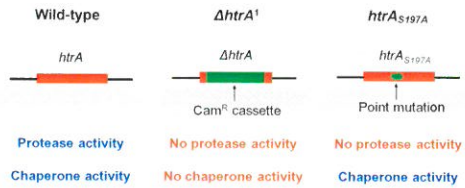
Chaperone activity *in vitro* was defined as the ability of proteolytically inactive HtrA ($HtrA_{S197A}$) to prevent aggregation of denatured lysozyme.

Lysozyme was denatured with DTT and aggregate formation was followed by light (360nm) scattering.

The molar ratio of lysozyme to HtrA was 2:1

htrA mutants studied

We determined the contribution of chaperone and protease activity, respectively, to heat and oxidative stress tolerance of *C. jejuni*, by comparing the phenotypes of three isogenic strains:



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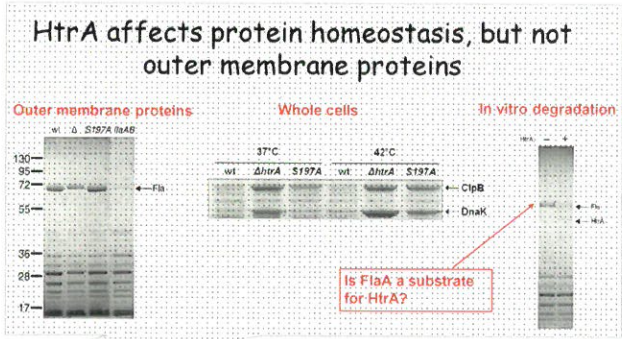
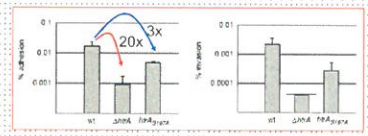
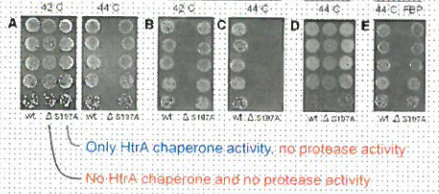
HtrA affects protein homeostasis, but not outer membrane proteins



HtrA is important for adherence to and invasion of INT-407cells



Heat sensitivity of *htrA* mutants depends on the oxygen level



htrA mutants are sensitive to ROS, but survives well in macrophages

Disc diffusion assay

Temp.	Oxidative stress agent	Inhibition zone ± SD (mm) Wild-type	*ΔhtrA*[a]	*htrA*$_{S197A}$[a]
37°C	H_2O_2	68 ± 5.3	81 ± 7.1**	79 ± 11*
	Cumene hydroperoxide	76 ± 6.9	88 ± 10*	88 ± 8.2*
	Paraquat	58 ± 4.6	70 ± 2.2**	66 ± 5.2
42°C	H_2O_2	59 ± 5.9	77 ± 5.7***	72 ± 7.0**
	Cumene hydroperoxide	73 ± 6.6	91 ± 6.9***	91 ± 11**
	Paraquat	50 ± 2.4	64 ± 1.4***	63 ± 2.0***

Survival in macrophages